138: 5' -GTGGTGGTTATGCCGATCGC-3' (SEQ ID No:26)

B7 WPR2: 5'-TAAGAGGCCTATAAGAGGCGG-3' (SEQ ID No:27)

924: 5'-AAGTCAGCCCAGAGGAGACT-3' (SEQ ID No:28)

➢ On Page 110, please replace the paragraph at lines 21-24 with the following text:

Degenerate oligonucleotides *hh*5.1 (SEQ ID No:30) and *hh*3.3 (SEQ ID No:31) were used to amplify genomic zebrafish DNA

B8 *hh* 5.1: AG(CA)GITG(CT)AA(AG)GA(AG)(CA)(AG)I(GCT)IAA (SEQ ID No:30)

hh 3.3: CTCIACIGCIA(GA)ICK=(GT)IGCIA (SEQ ID No:31)

The replacement paragraphs presented above incorporate changes as indicated by the marked-up versions below.

Figure 1 represents the amino acid sequences of two chick *hh* clones, chicken *hedgehog*-A (pCHA; SEQ ID No:35) and chicken *hedgehog*-B (pCHB; SEQ ID No:36). These clones were obtained using degenerate primers corresponding to the underlined amino acid residues of the Drosophila sequence (SEQ ID No:47) (corresponding to residues 161-232 of SEQ ID No:34) also shown in Figure 1, followed by nested PCR using chicken genomic DNA.

Figure 5A is a "pileup" alignment of predicted amino acid sequences which compares Drosophila *hh* (D-hh; SEQ ID No:34), mouse *hh* (M-*Dhh*; SEQ ID No:9; M-*Ihh*; SEQ ID No:10; M-*Shh*; SEQ ID No:11), chicken *hh* (C-*Shh*; SEQ ID No:8), and zebrafish *hh* (Z-*Shh*; SEQ ID No:12). The predicted hydrophobic transmembrane/signal sequences are indicated in italics and the predicted signal sequence processing site is arrowed. The positions of introns interrupting the Drosophila *hh* and M-*Dhh* open reading frames are indicated by arrowheads. All amino acids shared among the six predicted *hh* proteins are indicated in bold. Figure 5B is a sequence alignment of the N-terminal portion of vertebrate *hedgehog* proteins (SEQ ID Nos:48-54), and the predicted degenerate sequence "CON" (SEQ ID No: 41).

Figures 9A and 9B illustrate the comparison of zebrafish *Shh* (Z-*Shh*) and Drosophila *hh* (hh) amino acid sequences (SEQ ID Nos:12 and 34). Figure 9A is an alignment of zebrafish *Shh* and Drosophila *hh* amino acid sequences. Identical amino acids are linked by vertical bars. Dots indicate gaps introduced for optimal alignment. Putative transmembrane/signal peptide sequences are underlined (Kyte and Doolittle (1982) *J Mol Biol* 157:133-148). The position of exon boundaries in the Drosophila gene are indicated by arrowheads. The region of highest similarity between Z-*Shh* and *hh* overlaps exon 2. Figure 9B is a schematic comparison of Z-*Shh* and drosophila *hh*. Black boxes indicate the position of the putative transmembrane/signal peptide sequences. relative to the amino-terminus. Sequence homologies were scored by taking into account the alignment of chemically similar amino acids and percentage of homology in the boxed regions is indicated.

Figure 10 (SEQ ID Nos:37 and 15-17) is an alignment of partial predicted amino acid sequences from three different zebrafish *hh* homologs. One of these sequences corresponds to *Shh*, while the other two define additional *hh* homologs in zebrafish, named *hh*(a) and *hh*(b). Amino acid identities among the three partial homologs are indicated by vertical bars.

All standard cloning techniques were performed according to Ausubel et. al. (1989), and all enzymes were obtained from Boehringer Mannheim Biochemicals. Degenerate oligonucleotides corresponding to amino acid residues 161 to 237 of the Drosophila *hedgehog* protein (SEQ ID No:34) (Lee, J.J. et. al., (1992) *Cell* 71: 33-50) were synthesized. These degenerate oligonucleotides, vHH5O (SEQ ID No:18), vHH3O (SEQ ID No:19), and vHH3I (SEQ ID No:20) also contained Eco RI, Cla I, and Xba I sites, respectively, on their 5' ends to facilitate subcloning. The nucleotide sequence of these oligos is given below:

vHH5O: 5'-GGAATTCCCAG(CA)GITG(CT)AA(AG)GA(AG)(CA)(AG)I(GCT)IAA-3' (SEQ ID No:18)
vHH3O: 5'-TCATCGATGGACCCA(GA)TC(GA)AAICCIGC(TC)TC-3' (SEQ ID No:19)
vHH3I: 5'-GCTCTAGAGCTCIACIGCIA(GA)IC(GT)IGC-3' (SEQ ID No:20)

where I represents inosine. Nested PCR was performed by first amplifying chicken genomic DNA using the vHH5O and vHH3O primer pair and then further amplifying that product using the vHH5O and vHH3I primer pair. In each case the reaction conditions were: initial denaturation at 93° C for 2.5 min., followed by 30 cycles of 94° C for 45 s, 50° C for 1 min., 72° C for 1, and a final incubation of 72° C for 5 min. The 220 bp PCR product was subcloned into pGEM7zf (Promega). Two unique clones, pCHA (SEQ ID No:35) and pCHB (SEQ ID No:36) were identified.

Oligonucleotide sequences are as follows:

lacI: 5'-AGCTGTCGACGCGGCCGCTACGTAGGTTACCGACGTCAAGCTTAGATCTC-3' (SEQ ID No:21)

lac2: 5'-AGCTGAGATCTAAGCTTGACGTCGGTAACCTACGTAGCGGCCGCGTCGAC-3' (SEQ ID No:22)

Sf-1: 5'-GATCGGCCAGGCAGGCCTCGCGATATCGTCACCGCGGTATTCGAA-3' (SEQ ID No:23)

Sf-2: 5'-AGTGCCAGTCGGGGCCCCCAGGGCCGCGCC-3' (SEQ ID No:24)

Oligonucleotide sequences are as follows:

137: 5'-TACCACAGCGGATGGTTCGG-3' (SEQ ID No:25)

138: 5' -GTGGTGGTTATGCCGATCGC-3' (SEQ ID No:26)

WPR2: 5'-TAAGAGGCCTATAAGAGGCGG-3' (SEQ ID No:27)

924: 5'-AAGTCAGCCCAGAGGAGACT-3' (SEQ ID No:28)

Degenerate oligonucleotides *hh*5.1 (SEQ ID No:30) and *hh*3.3 (SEQ ID No:31) were used to amplify genomic zebrafish DNA

hh 5.1: AG(CA)GITG(CT)AA(AG)GA(AG)(CA)(AG)I(GCT)IAA (SEQ ID No:30)

hh 3.3: CTCIACIGCIA(GA)ICK=(GT)IGCIA (SEQ ID No:31)

Although Applicants believe no fees are due, the Commissioner is hereby authorized to charge any deficiency in the fees filed, asserted to be filed or which should have been filed herewith (or with any paper hereafter filed in this application by this firm) to our Deposit Account No. 18-1945, under Order No. HMSU-P14-006. Please direct any questions arising from this submission to the undersigned at (617) 951-7794.

Respectfully Submitted,

Date: January 17, 2003

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